Welcome to BIOS 318, Microbiology Laboratory

The 30,000 or so species of bacteria that have been identified by microbiologists[1] represent a very tiny fraction of the estimated number of bacterial species on Earth. Applying ecological scaling laws to data from over 35,000 surveys of microbial populations, researchers have estimated that from 100 billion to 1 trillion species of bacteria, archaea, and fungi live in our soil, in oceans, on animals and plants, etc.[2] This means that for every bacterial species successfully isolated from an environmental sample, as many as 10,000 species remain undetected. If the one trillion estimate is accurate, then if 100 Rice students *each* discover 100 new species every year (obviously not a realistic expectation), it will take 100 million years to discover all of them.

Our project

As a class we will attempt to isolate and identify species of *Streptomyces* bacteria, to support some very cool studies that are currently under way in the Shamoo lab, Department of BioSciences, Rice University.

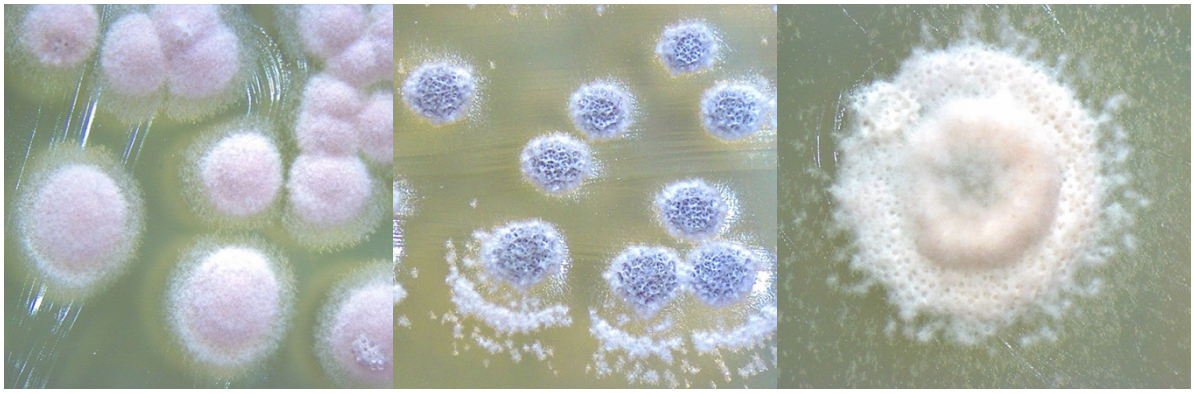

Fig. 1. (left to right) colonies of isolate T8-JL-3; colonies of isolate T5-ML-1, and a single colony of isolate T7-NS-2, all from the class of fall 2020. Photos by Lauren Eveland, BioSciences Dept.

[1] Dykhuizen, D. "Species numbers in Bacteria." Proc Calif Acad Sci. 2005 Jun 3; 56(6 Suppl 1): 62–71.
[2] Offord, Catherine. "Earth: Home to 1 Trillion Microbial Species." The Scientist (2016): http://www.the-scientist.com/?articles.view/articleNo/46016/title/Earth--Home-to-1-Trillion-Microbial-Species/

It has been proposed that positive and negative interactions among bacterial strains define much of the ecology of soil. The Shamoo lab is studying the evolution of such interactions. *Streptomyces* are the source of the majority of antibiotics in use today, thus a possible side benefit of this research is the discovery of new antimicrobial substances. It's a long shot, but quite possible.[3]

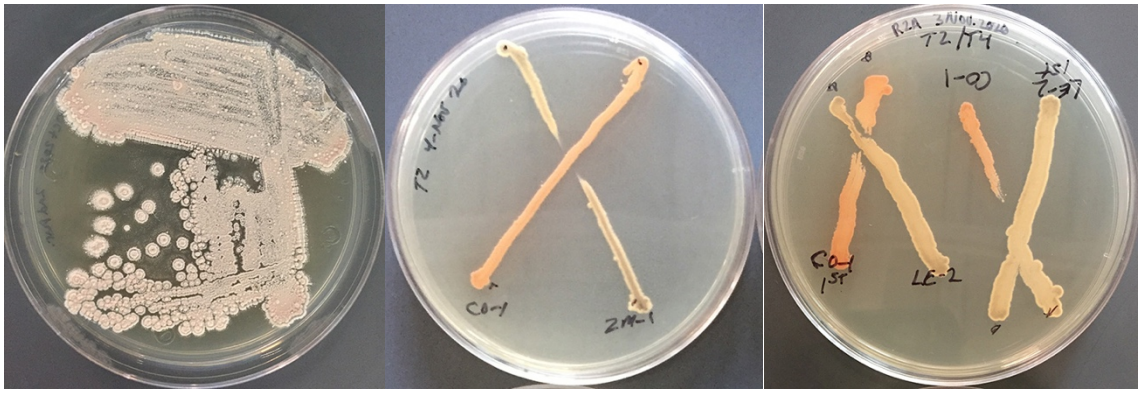

Fig. 2. (left) Dilution streak plate of isolate T7-NS-2; (middle and right) streak plates testing for competitive interactions between isolates. Photos by Lauren Eveland, BioSciences Dept.

Each team of students will provide a soil sample from which to isolate *Streptomyces* cultures. After identifying potential *Streptomyces* colonies you* will propagate isolates to obtain pure cultures and characterize them by phenotype and through 16S rRNA sequence analysis.

*Due to COVID restrictions it may be necessary to limit the hands-on work to the instructor and laboratory manager. Some students may be able to participate in laboratory activities, depending on the infection rates closer to the start of the course.

Objectives and learning outcomes

The main goal of this course, as must be true for any experiential course, is personal development. We want you to build on skills that you've already acquired and learn/apply new ones. Below we summarize our most important learning objectives and outcomes.

1. Learn to work as a productive member of a research team on a long term project
2. Be able to access and employ published reference materials, current primary literature, and other information sources in order to advance

[3] https://phys.org/news/2018-12-bacteria-ancient-irish-soil-halts.html

your understanding of the properties of bacteria and how to isolate and characterize them
3. Learn to integrate your previous and newly acquired experience into development of new techniques and strategies
4. Improve your powers of observation, such as the ability to use physical and metabolic differences to distinguish strains and species of bacteria and to draw information from microscopic observations
5. Learn to make the decisions that are necessary in order to conduct a systematic open-ended investigation
6. Learn to make your work accessible to yourself and others by documenting your activities and findings, by contributing to our laboratory's database, and by summarizing your work in a clearly written report
7. Learn to appreciate the value and potential of pure science, that is, the pursuit of knowledge for its own sake
8. Acquire proficiency in essential methodologies and use your newly acquired skills to advance our project

Because this semester's class is 100% remote, we will are unlikely to address an eighth learning objective, namely to learn and practice good laboratory "citizenship," including respect for people, the facility, and equipment.

Please see the Syllabus for course details, course timetable, and a list of assignments with due dates.

BIOS 318 Spring 2021 Syllabus

Welcome again to our microbiology laboratory. Here you will find information about the course, instructors, and policies, and a timetable of events. Details are subject to change at the instructor's discretion.

Mode of delivery

We are planning for remote participation via Zoom, with meetings at 1:30 pm on your scheduled lab days (MW Mar 17 through Apr 28 for section 901, TTh Mar 18 through Apr 29 for section 902). Please block out the scheduled times on your calendar. You must not schedule events that conflict with class meetings, nor let anyone schedule a conflicting event for you.

Depending on the COVID numbers there may be opportunities for direct participation by a limited number of students on campus.

Instructor and office hours

Instructor: David R. Caprette, Ph.D.
Teaching Professor, BioSciences, caprette@rice.edu

Office hours will be by appointment, arranged via email or requested during class. I will be available for remote meetings following most classes.

Preparing for the course

Please plan to apply the skills and best practices that you learned in BIOS (BIOC) 211, Intermediate Experimental Biosciences (course prerequisite) and in BIOS (BIOC) 311, Advanced Experimental Biosciences, if you have had the opportunity to complete that course. You will not need PPE or a laboratory notebook for the fall 2020 version of this course.

Prior to the first day

- Contact your team members and arrange for meetings outside of class time. Team assignments will be announced March 1.
- Assign one team member responsibility for collecting a soil sample, to be delivered to the laboratory classroom by the morning of March 15.
- Review the information in the file Streptomyces protocols.pptx. This file outlines our strategy for obtaining isolates from soil samples.

Schedule of activities

Although the course is set to start March 15/16, March 16 is a "sprinkle day" with no classes scheduled. Therefore we will have our first meetings March 17 & 18.

Beginning on the first day and throughout the course, maintain a personal journal documenting your participation in the course, including activities outside of scheduled class meetings. Keep notes as you would for a hands-on laboratory. Supplement your journal with outside research you have conducted, notes from websites and journal articles, analysis that you conduct outside of class, etc. Periodically post your journal in Canvas (Individual journal assignment).

Each team is to maintain separate documentation of its activities. This documentation should be a summary of the combined efforts of all team members, including assigned responsibilities, frequently updated summaries of data and observations, summaries of meetings, shared ideas, reflections shared by the entire team, etc. This document is to be posted in Canvas in the assignment "Team journal."

You are strongly encouraged to update your documentation frequently.

<u>Weds/Thurs Mar 17/18</u>

- Introduction to the project, including motivation for the research, our role in the project, planned work, and how your performance will be assessed
- Review the presentation "Streptomyces protocols.pptx"
- Dilute soil samples, prepare and incubate spread plates for isolating colonies

<u>Mon/Tues Mar 22/23</u>

- Examine spread plates and select plates from which to isolate colonies
- With the aid of a dissecting microscope, identify and mark probable *Streptomyces* colonies
- Label and inoculate plates with colony material, using a dilution streak method

<u>Weds/Thurs Mar 24/25</u>

- Examine streak plates, characterize each isolate by colony phenotype (color of spore mass, color of substrate mycelium, odor, other)

- Prepare smears of colony material for Gram staining
- Prepare wet mounts for microscopy of living material
- Examine wet mounts, look for evidence of cell morphology typical of *Streptomyces*
- Conduct the Gram stain procedure
- Examine Gram stains in a compound microscope to confirm cell morphology typical of *Streptomyces*

Prior to the next class meeting

- Select one or two isolates for characterization via 16S rRNA analysis

Mon/Tues Mar 29/30

- Review genomic DNA isolation & pcr method
- Discuss a sample agarose gel; how to recognize a good pcr product
- Examine a complete 16S rRNA sequence from NCBI database
    - How to access the database for a FASTA sequence
    - Locations of primer sequences (fd1, rp1, Strep primers, and more) and variable regions on a map prepared in an Excel spreadsheet
- Examine sample chromatograms (Finch TV); review how to assess quality, clean up (if necessary), copy and paste into a Word file.
- Converting sequences generated with reverse primers (reverse/complement tool).
- Using BLAST to align two or more sequences
- Assembling a sequence from multiple pcr runs
- Using BLAST to identify similar sequences from the 16S rRNA database

Prior to the next class meeting (in one week)

In order to help you prepare to characterize your own isolates, your team will be provided with data and observations for two isolates that were obtained in fall 2020. You will be given a list of primers used, chromatograms generated, observations made on colony phenotype, and representative photographs of colony material. Use these resources as follows:

- Prepare a text file of FASTA sequences from the chromatogram files
- Convert the sequences generated by the reverse primers using the reverse/complement tool
- Use the BLAST alignment tool to systematically align sequences in order to assemble as complete sequence as you can for the isolate
- Use the BLAST search tool to identify highly similar sequences

- Have the results ready to present to the class – were you able to narrow the list of probable species to one or a few?

Mon/Tues Apr 5/6

- Teams present their findings for their assigned isolates
- Introduction to Bergey's Manual of Systematic Bacteriology, Second Edition, Volume V – The Actinobacteria; where to find information on Genus *Streptomyces*, species descriptions, etc.
- By this time we will have conducted genomic DNA isolation and pcr on your selected isolates; chromatogram files will be made available

Remaining meetings and final assignment

I will open a Zoom session at 1:30 pm each class day through the end of the course. Note that April 8 is a "sprinkle day" with no classes. I will be available for consultation and questions on the project work.

Your final assignment is a team-written paper on your project work. The Canvas assignment "Team write-up" describes the details.

Course grade

Preparation and performance (30% of the course grade) will be determined from meeting attendance and participation in the project work, assessed by examining your personal journal and team journal entries and the acknowledgements section of the Team write-up. The remaining 70% of your grade will come from the Team write-up.

Additional information and policies

Absences. You will be expected to participate in all scheduled Zoom meetings unless otherwise advised. Missing meetings without a legitimate excuse will result in a deduction from your lab preparation and performance grade.

Lab safety. This policy does not apply to the remote version, however if you catch one of us violating safety rules then let me know. You may earn bonus credit.

Late assignments. An assignment that is submitted late will lose 10% per day including holidays and weekends.

Honor code policy. As with all biosciences courses, the Rice University Honor Code applies to all personal conduct and assignments. All work submitted under your name must be your own work, not copied from another source nor completed with unauthorized assistance. Compliance with the honor code includes providing accurate information when requested, such as honest evaluations of yourself and your teammates.

Students with disabilities. If you have a documented disability that may affect academic performance, you should: 1) make sure this documentation is on file with Disability Resource Center (Allen Center, Room 111 / adarice@rice.edu / x5841) to determine the accommodations you need; and 2) meet with me to discuss your accommodation needs.